EFFECT OF Ca^{++} AND GABA ON OXIDATION OF GLUTAMATE AND GLUTAMINE BY RAT BRAIN SYNAPTOSOMES

N. S. Nilova

UDC 577.17.49:577.17

Experiments in vitro showed that the addition of Ca^{++} inhibits respiration of rat brain synaptosomes in the presence of glutamate and glutamine. The addition of GABA potentiates the inhibitory effect of Ca^{++} on the oxidation of glutamine but not of glutamate. GABA itself has no effect on the oxidation of either glutamate or glutamine.

KEY WORDS: synaptosomes; oxidation; glutamine; glutamate; γ-aminobutyric acid.

Regulation of the glutamic acid system, including glutamate, glutamine, ammonia, aspartate, and γ-aminobutyric acid (GABA), is of essential interest in neurochemistry, for each of its components is directly linked with the function of nerve tissue. The glutamic acid system is a single metabolic entity, in a state of dynamic equilibrium [5]. The modulating role of Ca^{++} and GABA in nerve tissue is very diverse. Ca^{++} participates in conduction of the nervous impulse [1] and can also cause conformational changes in various intracellular membranes and it acts on transport processes through its influence on the state of carriers [2]. The intracellular effects of GABA as a biologically active compound may also be connected with changes in the conformation and permeability of various cell membranes.

The object of this investigation was to study the combined effect of Ca^{++} and GABA on oxidation of glutamate and glutamine by brain synaptosomes.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 160-180 g. The animals' brains were removed entirely except for the cerebellum. Synaptosomes were obtained by layering the total mitochondrial fraction suspended in 0.3M sucrose on 0.8M sucrose [8]. Respiration of the synaptosomes was recorded in a Warberg apparatus. Krebs–Ringer solution was used as the incubation medium, with glutamate or glutamine in a concentration of 20 mM as the respiration substrate. The protein concentration in the samples was 7-9 mg. Protein was determined by Lowry's method [10]. Glutaminase activity was determined from the quantity of ammonia liberated from glutamine during incubation of the samples for 30 min at 37°C. The ammonia concentration was determined by a microdiffusion method [3].

EXPERIMENTAL RESULTS

The effect of Ca^{++} in different concentrations and also the combined effect of Ca^{++} and GABA on the oxidation of glutamate and glutamine by rat brain synaptosomes were investigated. It will be clear from Table 1 that the addition of Ca^{++} in concentrations of 0.75, 1.5, and 3.0 mM reduced the oxygen consumption by 12-16% when glutamate was used. This inhibition of respiration was equal for Ca^{++} in all concentrations used. The simultaneous addition of Ca^{++} and GABA (3 mM) did not change the character of the effect of this cation: inhibition of respiration during utilization of glutamate was of the same magnitude (11-13%). As regards respiration with glutamine, in this case Ca^{++} in a concentration of 75 mM did not affect the oxygen consumption, in a concentration of 1.5 mM it inhibited respiration statistically significantly by 9%, and in a concentration of 3.0 mM, by 13%. In this case a statistically significant strengthening of the effect of Ca^{++} was observed on an increase in its concentration in the incubation medium. The addition of GABA in a concentration of 3 mM caused an increase in the effect of Ca^{++}: the inhibitory effect of the cation was manifested

Laboratory of Functional Neurochemistry, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 9, pp. 293-295, September, 1978. Original article submitted November 10, 1977.

0007-4888/78/8609-1158$07.50

TABLE 1. Respiration (in μmoles O_2/10 mg protein/h) of Rat Brain Synaptosomes in Presence of Ca^{++} and GABA, M ± m

Ca^{++} concentration, mM	Glutamine (20 mM)				Glutamate (20 mM)			
	without Ca^{++}	Ca^{2+}	GABA (3 mM)	Ca^{++} + GABA (3 mM)	without Ca^{++}	Ca^{2+}	GABA (3 mM)	Ca^{++} + GABA (3 mM)
0,75	1,99±0,06 (11)	1,96±0,08 (11)	1,92±0,07 (12)	1,70±0,07* (12)	2,25±0,04 (21)	2,08±0,05* (22)	2,05±0,08 (10)	1,83±0,07* (12)
1,5	1,99±0,06 (11)	1,81±0,05* (10)	2,04±0,04 (15)	1,72±0,03* (15)	2,25±0,04 (21)	1,86±0,04* (22)	2,29±0,04 (15)	2,02±0,04* (15)
3,0	1,99±0,06 (11)	1,73±0,06* (11)	2,04±0,03 (15)	1,62±0,02* (15)	2,25±0,04 (21)	1,93±0,04* (22)	2,23±0,03 (15)	1,93±0,03* (15)

Legend. 1. Asterisk indicates $P < 0.05$ compared with corresponding control (without Ca^{++}). 2. Number of experiments shown in parentheses.

TABLE 2. Effect of Ca^{++} in Presence of GABA on Glutaminase Activity (in μg N/mg protein/30 min) of Rat Brain Synaptosomes, M ± m

Enzyme	GABA (3 mM)	Ca^{++} (0.75 mM) + GABA (3 mM)	GABA (3 mM)	Ca^{++} (3 mM) + GABA (3 mM)
Glutamine transaminase	3,2±0,18 (9)	4,1±0,14* (12)	3,2±0,16 (8)	5,0±0,59* (12)
Phosphate-independent (maleate-activated) glutaminase	18,5±2,10 (12)	25,0±2,07* (12)	22,7±0,76 (12)	25,3±0,50* (12)
Phosphate-dependent glutaminase	12,1±0,48 (11)	12,1±0,32 (12)	12,1±0,25 (12)	12,9±0,28* (12)**

Legend. Two asterisks indicate that in this experiment Ca^{++} was used in a concentration of 1.5 mM. Remainder of legend as in Table 1.

in a concentration as low as 0.75 mM, and the effect was rather greater in concentrations of 1.5 mM (16%) and 3.0 mM (21%).

A separate series of experiments showed that GABA by itself did not affect the oxidation of glutamate and glutamine by synaptosomes. For instance, respiration during utilization of glutamate (20 mM) in the absence of GABA was (in μmoles O_2/10 mg protein/h) 2.04 ± 0.06, and on the addition of 3 mM GABA it was 2.14 ± 0.04 (n = 12). Similarly, during utilization of glutamine in a concentration of 20 mM the oxygen consumption was 1.71 ± 0.06 in the absence of GABA and 1.73 ± 0.05 μmoles O_2/10 mg protein/h in the presence of 3 mM GABA.

To ascertain whether the potentiation of the inhibitory action of Ca^{++} in the presence of GABA is connected with additional inhibition of glutaminases, the glutaminase activity of the rat brain synaptosomes was investigated in the presence of GABA and during combined incubation with Ca^{++} and GABA. As Table 2 shows, addition of Ca^{++} in concentrations of 0.75 and 3.0 mM to samples containing 3 mM GABA caused a statistically significant increase in glutamine transaminase and phosphate-independent glutaminase activity compared with samples of synaptosomes containing GABA only. There was little change in phosphate-dependent glutaminase activity. The results thus indicate that during the combined action of Ca^{++} and GABA no additional inhibition of the glutaminase was observed and, consequently, that the strengthening of the inhibitory effect of Ca^{++} in the presence of GABA on glutamine oxidation does not take place at the stage of glutaminase reactions.

The inhibitory action of Ca^{++} on the oxidation of glutamate and glutamine may take place both at the level of oxidative processes in the synaptic mitochondria and at the level of supply of the substrates to the synaptosomes. It has been shown that Ca^{++} in mitochondria can inhibit the oxidation of NAD^+-dependent substrates by binding pyridine nucleotides [7, 12]. Possibly the identically oriented inhibitory effect of Ca^{++} on the oxidation of glutamate and glutamine was primarily due to this action of Ca^{++} at the level of oxidative processes in the mitochondria. At the same time, some difference was found in the action of Ca^{++} on the oxidation of glutamate and glutamine in these experiments. By contrast with glutamate, during utilization of glutamine the inhibitory effect of Ca^{++} increased with an increase in the concentration of the cation in the medium; furthermore, potentiation of the effect of Ca^{++} on glutamate oxidation was observed during the combined use of Ca^{++} and GABA. These differences in the effect of Ca^{++} may be connected with its possible action on glutamine transport into the synaptosomes.

The existence of active Na-dependent transport with high affinity and passive Na-independent transport with low affinity has been demonstrated for the supplying of glutamate to synaptosomes [6, 9]; for glutamine

in the synaptosomes only a passive transport system has been found [4, 11]. If the supply of glutamine to the synaptosomes is in fact not controlled by the Na^+ concentration in the external medium, the importance of other factors in the control of transport may be increased. In particular, some action of the combined addition of Ca^{++} and GABA to the medium on the glutamine supply is possible.

LITERATURE CITED

1. R. N. Glebov and G. N. Kryzhanovskii, Usp. Fiziol. Nauk, 6, 3 (1975).
2. S. V. Levin, Structural Changes in Cell Membranes [in Russian], Leningrad (1976).
3. A. I. Silakova, G. P. Trush, and A. Yavilyakova, Vopr. Med. Khimii, 8, 538 (1962).
4. R. J. Baldessarini and C. Yorke, J. Neurochem., 23, 839 (1974).
5. A. M. Benjamin and J. H. Quastel, J. Neurochem., 25, 197 (1975).
6. J. P. Bennett and S. H. Snyder, J. Neurochem., 21, 1533 (1973).
7. P. Gazzotti, Biochem. Biophys. Res. Commun., 67, 634 (1975).
8. F. Hajos, Brain Res., 93, 485 (1975).
9. W. J. Logan and S. H. Snyder, Brain Res., 42, 413 (1972).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
11. P. J. Roberts and P. Keen, Brain Res., 67, 352 (1974).
12. A. Vinogradov, A. Scarpa, and B. Chance, Arch. Biochem., 152, 646 (1972).

ACTION OF ACUTE CARDIAC ISCHEMIA ON ACTIVITY OF THE PROTEIN-SYNTHESIZING SYSTEM OF THE INNER MITOCHONDRIAL MEMBRANES OF THE MYOCARDIUM

L. A. Kopteva, L. I. Zhevalyukova, and V. I. Biryuzova

UDC 616.12-005.4-036.11-07: 616, 127-008.939.6-074

Acute cardiac ischemia caused by ligation of the descending branch of the left coronary artery leads to a sharp increase in the activity of the protein-synthesizing system of the inner mitochondrial membranes of the myocardium throughout the period of organization of the experimental infarct, as reflected in an increase in the synthesis of protein and RNA in the mitochondria. During development of the infarct considerable changes are observed in the ultrastructure of the inner mitochondrial membranes of the myocardium, the degree of which is directly dependent on the stage of development of the pathological process.

KEY WORDS: mitochondria of the heart; ischemia; protein and RNA synthesis; ultrastructure.

The writers showed previously that necrotic lesions of the heart have a marked effect on the activity of protein and RNA synthesis in the inner membranes of the mitochondria of the undamaged parts of the myocardium [2-4]. However, the question arose whether these results reflect a true effect of ischemia or whether they may be in part the result of the effect of the sympathomimetic drug isoproterenol, which was used to create the necrotic foci in the myocardium.

In the present investigation classical ischemia of the myocardium caused by ligation of the descending branch of the left coronary artery was used as the experimental model.

EXPERIMENTAL METHOD

Male Wistar rats weighing 250-280 g were used. Since the development of the experimental myocardial infarct in the animals continued for about three weeks, and since the typical picture of proliferation of con-

Institute of General Pathology and Pathophysiology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 9, pp. 295-297, September, 1978. Original article submitted March 2, 1978.